which have been well studied in the last decade, with *Microtus* species, which have been studied less intensively, in Fig. 2, b and c. *Peromyscus* shows a much better fit (r = .93) to the equation than does *Microtus* (r = .68), suggesting that poorly studied species serve only to increase the variance and do not introduce artificial correlations into the data.

In conclusion, the number of mite species using a rodent species is a function of the rodent's distributional area and, to a small degree, its latitude. The fit to the species-area equation reported here suggests that island biogeography theories are plausible explanations for the data observed, although conclusive evidence would require that species turnover rates for host "islands" be measured (*18*). For mites on mammals we believe the resulting species equilibrium numbers are determined by immigration and extinction rates which act primarily in evolutionary time. One would not expect that increasing area would greatly increase the colonization rate in ecological time for host-specific mite species.

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Leukemia Virus–Induced Immunosuppression: Scanning Electron Microscopy of Infected Spleen Cells

Abstract. *Spleen cells from mice infected with Friend leukemia virus were examined by scanning electron microscopy. Whereas splenocytes from normal noninfected animals showed the expected morphologic classes of lymphocytes, including those with smooth surfaces and with numerous villous projections, an alteration of cell type was evident within a few days after infection. Friend leukemia virus caused a rapid decrease in the number of villous cells, with a concomitant increase in the number of cells with smoother surfaces. By the end of the first 1 to 2 weeks after infection the majority of cells were smooth, many showing distinct morphologic changes, including "holes" and a spongy appearance. Nearly all of the splenocytes were abnormal in appearance by days 17 to 30 after infection, with most showing a spongy topography. These changes paralleled the marked immunosuppression induced by Friend leukemia virus infection, as well as the appearance of virus-associated surface antigen on individual splenocytes. Topographic changes evident by examination with scanning electron microscopy were not readily apparent by either standard histology or transmission electron microscopy.*

Infection of susceptible strains of mice with an oncornavirus such as Friend leukemia virus (FLV) invariably leads to a marked and generalized immunosuppression (*1*). However, the mechanism whereby tumor virus infection depresses immunologic competence is still not clear. Earlier studies in this and other laboratories suggested that leukemia viruses preferentially affect antibody precursor cells rather than antibody-forming cells per se (*2*). Cell transfer studies with splenocytes from leukemia virus–infected mice, as well as transfer of bone marrow or thymocytes (or both) from infected animals, focused attention on the B lymphocyte as the most likely target of leukemia virus–induced immunosuppression (*3*). Furthermore, immunohistologic studies revealed a marked decrease in cells bearing immunoglobulin

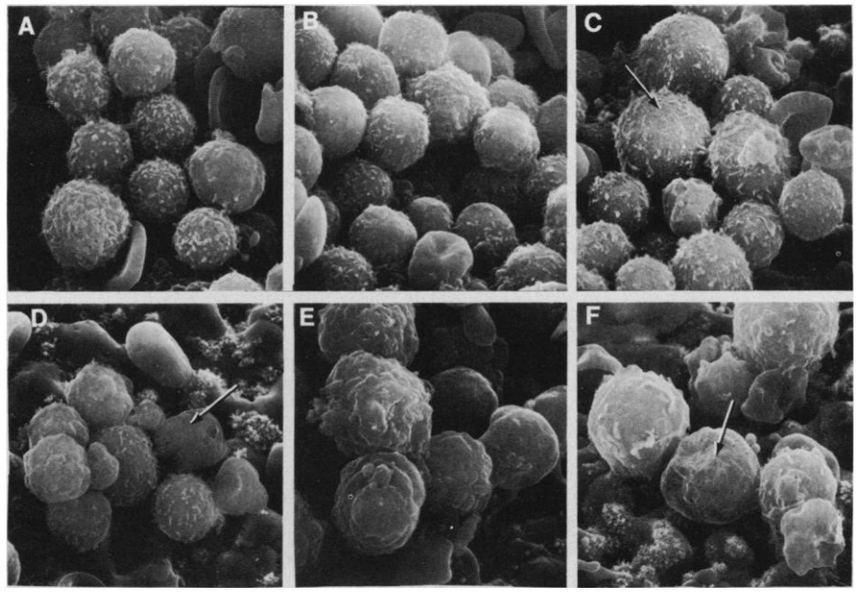

Fig. 1. Scanning electron micrographs of spleen cells. (A) Typical spleen cells from normal Balb/c mice showing villous and smooth cells; (B) spleen cells from 5-day infected mice showing moderate changes including fewer villous cells and one moderately "spongy" cell; (C) 7-day infected spleen cells, many of which are large and deformed with altered surface topography (arrow); (D) 10-day infected spleen cells with smoother surfaces and one highly spongy cell (arrow); (E) 17-day infected spleen cells most of which are large and deformed; (F) spleen cells from 30-day infected mice showing large, smooth surfaces, some with "holes" and spongy appearance (arrow) (× 2500).

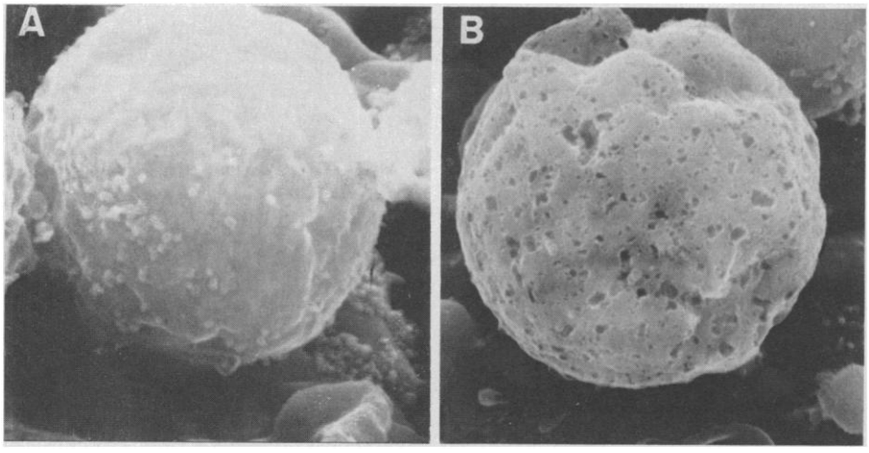

Fig. 2. Large smoother surfaced cells characteristic of FLV infection. (A) Cell with smooth surface, some villi, and occasional "holes"; (B) large cell with highly spongy surface characteristics 30 days after infection (× 7900).

surface receptors and a depression in the capping response of immunoglobulin positive cells prior to development of splenomegaly but concomitant with the initial development of immunosuppression (*4*). Both immunoglobulin surface receptors and capping are characteristic of B cells.

A previous study in this laboratory on the ultrastructure of antibody-forming tissue from FLV-infected mice (*5*) showed the presence of virus particles in immature blastlike lymphoid cells but not in plasmacytes which are generally considered the major cell type involved in antibody synthesis. However, subsequent studies showed virus particles budding directly from the surface of plasma cells actively secreting antibodies to sheep erythrocytes, indicating that virus infection and antibody formation by individual immunocytes were not mutually exclusive events (*6*). Recently, scanning electron microscopy has been utilized to differentiate B and T lymphocytes on the basis of surface topography (*7*). Although there is considerable overlap, it is generally felt that lymphocytes with numerous surface villous projections represent B lymphocytes, whereas T cells have many fewer villi or none at all when examined under appropriate conditions (*7*). Thus it seemed likely that examination of the spleen as well as other lymphoid tissue of FLV-infected mice by scanning electron microscopy might provide a valuable insight into the effects of leukemia virus infection on lymphocyte populations. Such changes might then be related to the infectious process per se and the development of immunosuppression.

For this study Balb/c mice were infected by intravenous injection of a stock FLV preparation (*2–4*). At various times thereafter representative mice were killed, and their spleen was obtained and minced into single cell suspensions, which were then processed by Ficoll-Hypaque centrifugation (*8*). The resulting lymphocyte-rich preparations were washed twice in McCoy's medium, fixed in 1 percent glutaraldehyde, and prepared for scanning electron microscopy examination by the method of Polliack (*7*). Samples were dried by the critical point method of Anderson (*9*), coated with gold paladium, and examined with an Etec autoscan microscope operating at 20 kv. In a parallel series of experiments representative animals from each group were immunized with sheep erythrocytes, and the number of antibody plaque-forming cells in the spleen of these animals was determined by the localized hemolytic assay in agar gel (*3, 8*). In addition, the spleen cell suspensions were examined by standard fluorescent microscopy for cells bearing surface immunoglobulin receptors, theta antigen, and FLV-induced tumor-associated antigen (*4*). At least 500 cells from duplicate spleen cell suspensions were examined to determine the percent of nucleated splenocytes with these surface markers.

Examination of spleen cells from normal mice for surface topography by scanning electron microscopy showed the expected spectrum of lymphoid cell types. As seen in Fig. 1A, most of the splenic lymphocytes from normal mice had numerous villous projections typical of B lymphocytes; lymphocytes without projections, presumably T cells, were also evident, but in smaller numbers. Many intermediate cell types with fewer villi were present, as were typical macrophages. Polliack *et al.*, studying normal B6 mouse spleen cells, reported recently that about 37 percent of the spleen cells were smooth and 47 percent villous, with the remainder of intermediate topography (*7*). Although this percent generally correlated with immunofluorescent studies, it should be noted that a smaller percentage of cells were stained with anti-immunoglobulin serum and anti-theta serum (*7*). The higher percentage of villous cells in normal Balb/c mice

Table 1. Changes in spleen cell populations in Balb/c mice after infection with FLV (Friend leukemia virus). Abbreviations: SRBC, sheep red blood cells; PFC, plaque-forming cells; sIg, surface immunoglobulin; SEM, scanning electron microscopy; ID_{50}, dose infectious to 50 percent of recipients.

Time in days after infection*	Average spleen weight (mg)	Number of nucleated spleen cells ($\times 10^7$)	Antibody response to SRBC (PFC per spleen)† ($\times 10^3$)	Percent lymphocytes staining for‡			SEM topography (percent)§		
				FLV antigen	Theta antigen	sIg	Smooth	Intermediate	Villous
Noninfected¶	147 ± 13	19.5 ± 3.6	98.5 ± 9.5	<1	34.5	38.2	18	12	70
2	155 ± 22	21.3 ± 4.5	86.1 ± 6.3	4.5	33.5	32.1			
5	185 ± 37	29.7 ± 3.6	42.5 ± 3.9	12.9	28.5	24.3	31	40	29
7	351 ± 62	34.5 ± 5.5	8.2 ± 1.5	38.5	27.3	21.1			
10	612 ± 110	42.6 ± 3.9	4.5 ± 1.1	76.2	19.5	14.3	29	60	11
17	1860 ± 350	81.8 ± 64	0.8 ± 0.3	83.5	8.3	5.2	45	49	6
25	2560 ± 865	157.8 ± 10.2	0.5 ± 0.2	88.6	6.2	2.5	99	<1	<1
30	2465 ± 918	196.9 ± 7.8	< 0.1 ± 0.1	94.5	4.2	<1			

*Groups of mice infected by intravenous injection of 1 : 100 dilution of FLV (50 to 100 ID_{50}) on day indicated before assay; five to ten mice tested at each time interval. †Average PFC response for five to ten spleens 4 days after intravenous immunization with 4×10^8 SRBC. ‡Average percent of spleen cells positive for indicated surface antigen when stained with fluorescein-labeled antiserums. §Average percent of spleen cells with indicated surface morphology as determined by SEM examination. ¶Controls.

in this study might have been due to strain differences or a difference in mouse age. However, the percentage of immunoglobulin and theta antigen positive cells from normal Balb/c mouse spleen cells indicated that there was a general correlation between cells classified by scanning electron microscopy and cells bearing globulin or theta antigen surface markers, similar to the study by Polliack *et al.* (*7*).

Within 5 days after infection with FLV a marked change in the percentage of the smooth and villous cell types was evident by scanning electron microscopy (Fig. 1B). An increasing number of cells appeared with smooth surfaces; these were generally larger than the typical smooth cells observed in normal mice. This type of cell increased rapidly during the next few days, so that by days 7 to 10 after infection 45 percent of the lymphoid cells were of this type (Fig. 1, C and D, and Table 1). By day 17 most splenocytes had a smooth topography (Fig. 1E and Table 1). Many of these smooth cells appeared to have "holes" in their surface; these cells first became apparent on days 5 and 10 and increased in number so that by day 30 numerous cells of this type were prominent (Fig. 2, A and B). These changes in cell type preceded the development of overt splenomegaly, which first became evident on days 7 to 10 and reached a maximum by 25 to 30 days after infection (Table 1). By this time most of the spleen cells were of the large, smooth-surfaced type, with few normal lymphocytes evident.

Immunosuppression preceded development of overt splenomegaly and the marked alteration of cell types shown by scanning electron microscopy (Table 1). However, FLV-associated antigen became evident in the spleen as early as 2 days after infection, and then increased rapidly so that a majority of the spleen cells showed FLV surface antigen by the tenth day after infection (Table 1). Similarly, the percentage of splenocytes containing surface immunoglobulin decreased as a function of time after infection. This decrease paralleled the decrease in number and percentage of highly villous cells in the spleen and was consistent with the likelihood that many villous cells represented B cells bearing surface immunoglobulin. There was a somewhat slower but still consistent decrease in the number and percentage of cells staining with antitheta serum during the course of infection (Table 1). It should be noted that these changes were not due merely to splenomegaly and dilution of "normal" spleen cells by tumor cells (Table 1).

The results of these studies are consistent with and extend earlier findings concerning histologic and ultrastructural features of lymphoid tissue from FLV-infected mice (*5, 6*). In those studies the marked immunodepression induced by FLV infection seemed temporally related to the morphologic alteration in the spleen, the organ where more than 95 percent of the antibody-forming cells appear in normal mice after a single intravenous or intraperitoneal injection of sheep red blood cells. The examinations with scanning electron microscopy in the present study further revealed rapid changes of cell types which are difficult to ascertain by direct histologic studies or transmission electron microscopy (*5, 6*).

The earlier studies had shown that cells rich in ribosomal particles and endoplasmic reticulum appeared within the first week after infection and, moreover, that many intracellular and surface particles characteristic of C-type virus were present in such cells. In the present study numerous large, smooth cells with a "spongy" surface punctuated by many holes were observed at the same time intervals after virus infection. It was not possible, however, to identify viruslike particles budding from these cells, even at higher magnifications (× 20,000). Nevertheless, the earlier transmission electron microscopy studies (*5, 6*), as well as fluorescent antibody studies (*4*), showed that a large percentage of cells in the spleen of FLV-infected mice contain virus-associated antigen. Thus the "holes" in the surface of the spleen cells might represent changes due to either virus replication or, alternatively, to immunologic injury due to antigen-antibody reactions on the surface of infected lymphocytes following a possible immune response of the host to the FLV antigen. However, further studies are necessary to determine whether an antivirus immune response early after infection induces these changes. Similarly, the relation between topographic changes revealed by scanning electron microscopy to other changes induced by leukemia virus infection, especially immunosuppression, require investigation.

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Differentiation of Red Blood Cells in vitro

Abstract. *Differentiation of red blood cells occurs in organ cultures of both liver and kidney tissue from tadpoles of the bullfrog* Rana catesbeiana. *Our evidence indicates that different red blood cell lines are produced by the two tissues and that these different cell lines contain different tadpole hemoglobins.*

Using organ cultures of kidney and liver tissue from tadpoles of the bullfrog *Rana catesbeiana*, we have evidence that these two erythropoietic tissues simultaneously produce different red blood cell lines containing different tadpole hemoglobins.

The larval stages of the bullfrog last for 1 to 2½ years, depending on how many winter seasons the animal must endure before reaching metamorphosis. During the larval period, the most obvious morphological changes are the overall growth of the animal and the development of hind legs. Three major and one or more minor types of hemoglobin are found in the circulating red blood cells (RBC's) during this period (*1*). Both the liver and the kidneys have been suggested as possible erythropoietic sites in the tadpole (*2*).

This report represents an important step in a series of studies that have progressed from in vivo to in vitro evaluation of sites of hemoglobin synthesis and erythropoiesis in bullfrog tadpoles. We first found that